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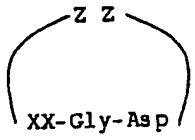
(54) Fibrinogen receptor antagonists.

(57) A fibrinogen receptor antagonist of the formula



wherein XX represents a synthetic alpha-amino acid containing a linear side chain and ZZ represents a sequence of 1, 2, 3 or 4 amino acids.

EP 0 422 938 A1

FIBRINOGEN RECEPTOR ANTAGONISTS

BACKGROUND OF THE INVENTION

This invention relates to compounds for inhibiting the binding of fibrinogen to blood platelets, and for inhibiting the aggregation of blood platelets.

Fibrinogen is a glycoprotein, present in blood plasma, which participates in platelet aggregation and fibrin formation. Platelets are cell-like anucleated fragments, found in the blood of all mammals, which participate in blood coagulation. Interaction of fibrinogen with a receptor on the platelet membrane glycoprotein complex IIb/IIIa is known to be essential for normal platelet function.

Zimmerman et al., U.S. Patent No. 4,683,291, describes peptides having utility in the study of fibrinogen-platelet, platelet-platelet, and cell-cell interactions. The peptides are described as having utility where it is desirable to retard or prevent formation of a thrombus or clot in the blood. The general formula for the peptides is:

H_2N-(Ch)-Arg-Gly-Asp-(Cx)-H

where Ch and Cx are sequences of amino acids.

Pierschbacher et al., U.S. Patent No. 4,589,881, describes the sequence of an 11.5 kDal polypeptide fragment of fibronectin which embodies the cell-attachment-promoting activity of fibronectin. A specifically described fragment is:

H-Tyr-Ala-Val-Thr-Gly-Arg-Gly-Asp-
Ser-Pro-Ala-Ser-Ser-Lys-Pro-Ile-
Ser-Ile-Asn-Tyr-Arg-Thr-Glu-Ile-
Asp-Lys-Pro-Ser-Gln-Met-OH

Ruoslahti et al., U.S. Patent No. 4,614.517, describes tetrapeptides which alter cell-attachment activity of cells to various substrates. The peptides are stated to "consist essentially of" the following sequence:

X-Arg-Gly-Asp-Ser-Y

wherein X is H or one or amino acids and Y is OH or one or amino acids. Figure 1 lists the polypeptides that were synthesized by Ruoslahti et al. in "determining the smallest peptide exhibiting cell attachment activity".

Ruoslahti et al., U.S. Patent No. 4,578,079, describes similar tetrapeptides having Ser substituted with Thr or Cys.

Pierschbacher et al., Proc. Natl. Acad. Sci. USA , Vol. 81, pp.5985-5988, October 1984 describe variants of the cell recognition site of fibronectin that retain attachment-promoting activity. They assayed the cell attachment-promoting activities of a number of structures closely resembling the Arg-Gly-Asp-Ser peptide, and found "that the arginine, glycine, and aspartate residues cannot be replaced even with closely related amino acids, but that several amino acids can replace serine without loss of activity."

Ruoslahti et al., Science , Vol. 238, pp. 491-497, October 23, 1987, discuss cell adhesion proteins. They specifically state that "[e]lucidation of the amino acid sequence of the cell-attachment domain in fibronectin and its duplication with synthetic peptides establish the sequence Arg-Gly-Asp (RGD) as the essential structure recognized by cells in fibronectin".

Cheresh, Proc. Natl. Acad. Sci. USA , Vol. 84, pp. 6471-6475, September 1987, describes the Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand Factor.

Adams et al., U. S. Patent No. 4,857,508, describes tetrapeptides which inhibit platelet aggregation and the formation of a thrombus. The tetrapeptides have the formula:

X-Gly-Asp-Y

wherein X can be H2NC(=NH)NH(CH2)nCH(Z)COOH or Ac-Arg, wherein Z = H, NH2, or NH-Acyl and n=1-4, and wherein Y can be Tyr-NH2, Phe-NH2 or a group of a specifically defined formula.

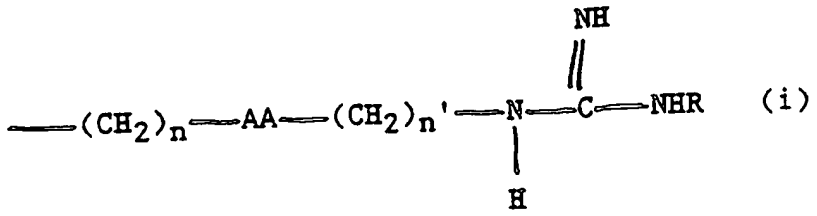
Applicants have discovered fibrinogen receptor antagonists which do not contain the amino acid sequence Arg-Gly-Asp which is taught in the art as specifically required for binding to platelet membrane glycoprotein complex IIb/IIIa.

SUMMARY OF THE INVENTION

Compounds of the present invention inhibit binding of fibrinogen to the platelet membrane glycoprotein complex IIb/IIIa receptor and contain an amino acid sequence:

XX:Gly-Asp

wherein XX is a synthetic alpha amino acid containing a linear side-chain.



(i)

or

$-(CH_2)_n-AA-(CH_2)_{n'}-NHR$ (ii)

wherein:

n is 1,2,3 or 4;

n′ is 2,3 or 4;

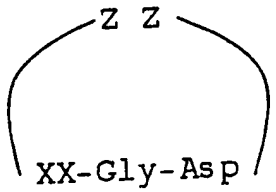
AA is an oxygen atom, a sulfur atom, or a single bond; and

R is H, C_{1-6} alkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylmethyl or substituted or unsubstituted cycloalkyl, provided that in case (i), when AA is a single bond and R is H, then $n+n'$ does not equal 1, 2, 3 or 4.

These compounds are surprising in view of the prior art which teaches that the sequence Arg-Gly-Asp is required in order to achieve binding to the IIb/IIIa receptor.

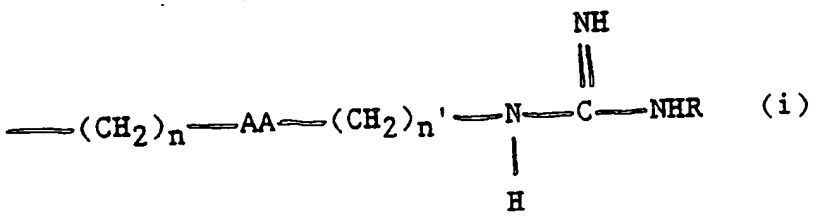
Preferred compounds of the invention are those having selectivity over other integrin receptors. The preferred compounds include those wherein XX is a synthetic alpha amino acid containing an amino group linear side chain, as represented above by (ii).

The present invention is a fibrinogen receptor antagonist having the following structure:



wherein XX represents a synthetic α-amino acid as defined below and ZZ represents a sequence of 1, 2, 3, or 4 amino acids as defined below.

XX shares an amide bond with Gly and an amide bond with ZZ, and is defined as having a side chain X



(i)

or

$-(CH_2)_n-AA-(CH_2)_{n'}-NHR$ (ii)

wherein:

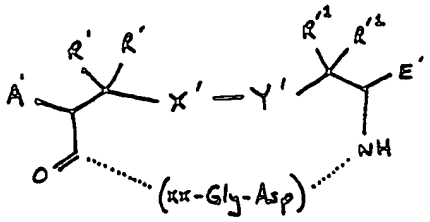
n is 1,2,3 or 4;

n′ is 2,3 or 4;

AA is an oxygen atom, a sulfur atom, or a single bond; and

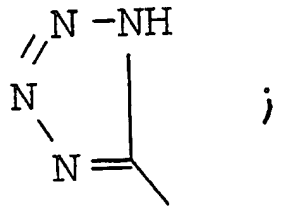
R is H, C_{1-6} alkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylmethyl or substituted or unsubstituted cycloalkyl, provided that in case (i), when AA is a single bond and R is H, then $n+n'$ does not equal 1, 2, 3 or 4.

Preferably, when X is defined by (i), then $n+n'$ is 3, AA is a single bond and R is phenyl or benzyl.
Preferably, when X is defined by (ii), then $n+n'$ is 5, AA is a single bond and R is H.
ZZ is defined as follows:



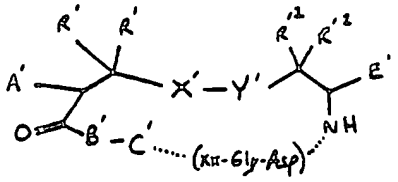
(I)

wherein:
A' is H, acylamido, acylaminoacylamido, acylamino-N-methylamino-acylamido;
R' and R'^1 are independently H, methyl, ethyl or a lower alkyl group having 1 to 5 carbons;
X'-Y' is S-S, CH_2-S, S-CH_2, CH_2CH_2, CH_2, $CH_2CH_2CH_2$, CH_2-S-S, CH_2-S-S-CH_2, S-S-CH_2; and
E' is H, COOH, $CONH_2$, $CONHR^2$, $CONR^3R^4$, CH_2OH,CO_2R^2·CH_3 wherein R^2 is an alkyl group having 1 to 4 carbon atoms, R^3R^4 is an alkyl group having 1 to 4 carbon atoms or NR^3R^4 is a secondary amino acid, or



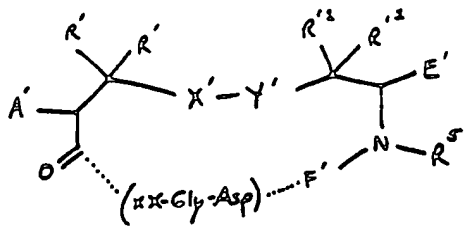
;

or ZZ is



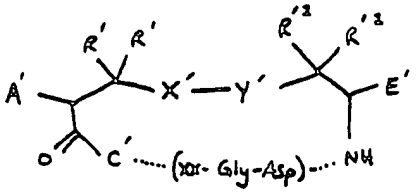
(II)

wherein:
A' is as defined above;
R' and R'^1 are as defined above;
X' - Y' is as defined above;
B' is a D- or L- α-amino acid;
C' is a D- or L- secondary α-amino acid preferably selected from proline, β- methylproline, β,β-dimethylproline, gamma-hydroxyproline, anhydroproline, thioproline, β - methylthioproline, β,β - dimethyl-thioproline, pipecolic acid, azetidine carboxylic acid and an N-methyl amino acid, or a D-or L- primary α-amino acid; and
E' is as defined above;
or ZZ is



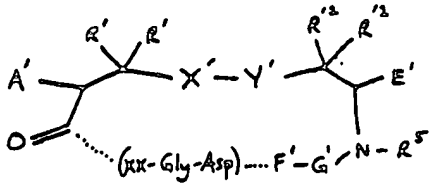
(III)

wherein:
A′ is as defined above;
R′ and R′¹ are as defined above;
X′ - Y′ are as defined above;
E′ is as defined above;
F′ is an L-amino acid, preferably an L-amino acid selected from tryptophan, phenylalanine, leucine, valine, isoleucine, α-naphthylalanine, β-naphthylalanine, methionine, tyrosine, arginine, lysine, homoarginine, ornithine, histidine, substituted tryptophan, substituted phenylalanine or substituted tyrosine; and R^5 is H or methyl;
or ZZ is



IV

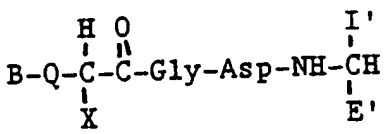
wherein
A′ is as defined above;
R′ and R′¹ are as defined above;
X′-Y′ is as defined above;
C′ is as defined above; and
E′ is as defined above.
or ZZ is



V

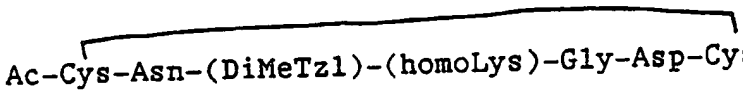
wherein
A′ is as defined above;
R′ and R′¹ are as defined above;
X′-Y′ is as defined above;
F′ is as defined above;
G′ is a D- or L-α-amino acid, secondary cyclic amino acid, or N-methyl amino acid;
E′ is as defined above; and
R^5 is as defined above.

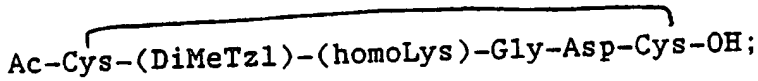
The present invention also is a fibrinogen receptor antagonist of the formula



wherein:
B represents zero, one or two substituted or unsubstituted amino acids;
Q represents H, NH, NH_2 or Ac-NH;
I′ represents a side chain of an amino acid defined by F′;
E′ is as defined above; and
X represents the side chain of amino acid XX as previously defined;
provided that when B is zero substituted or unsubstituted amino acids, then Q is H, NH_2 or Ac-NH, and that when B is one or two substituted or unsubstituted amino acids, then Q is NH.

Exemplary compounds of the invention are:
Ac-(Arg(Ph))-Gly-Asp-Phe;
Ac-(Arg(Bzl))-Gly-Asp-Phe;
Aha-Gly-Asp-Phe;
Aha-Gly-Asp-Trp;





(GuaValA)-Gly-Asp-Phe;
(GuaValA)-Gly-Asp-Trp;
(GuaHexA)-Gly-Asp-Trp;
(GuaHepA)-Gly-Asp-Trp;
(7-AhepA)-Gly-Asp-Trp;
(8-AoctA)-Gly-Asp-Trp;

Gly-Asp-Phe;

Ac-Cys-(homoLys)-Gly-Asp-Cys-OH;

Ac-Pen-(homoLys)-Gly-Asp-Cys-OH;

Ac-Cys-(Arg(Phenyl))-Gly-Asp-Cys-OH;

Ac-Cys-(Arg(Benzyl))-Gly-Asp-Cys-OH;

Ac-Cys-(homoLys)-Gly-Asp-Trp-Cys-OH;

Ac-Cys-(homoLys)-Gly-Asp-Trp-(N-MeCys)-OH;

Ac-Cys-Arg-(homoLys)-Gly-Asp-Cys-OH;

Ac-Cys-(homoLys)-Gly-Asp-Trp-Pro-Cys-NH_2;

c((7-AhepA)-(homoLys)-Gly-Asp-Trp-Pro);
c((6-AhexA)-(homoLys)-Gly-Asp-Trp-Pro);
c((7-AhepA)-(homoLys)-Gly-Asp-(beta-Nal)-Pro);
c((7-AhepA)-(Arg(Phenyl))-Gly-Asp-Trp-Pro);
c((7-AhepA)-(Arg(Benzyl))-Gly-Asp-Trp-Pro);

Ac-Cys-Asn-Pro-(homoLys)-Gly-Asp-Cys-OH;

Ac-Pen-Asn-(DiMeTzl)-(homoLys)-Gly-Asp-Cys-OH;

Ac-Cys-Asn-Pro-(Arg(Phenyl))-Gly-Asp-Cys-OH;

Ac-Cys-Asn-(DiMeTzl)-(Arg(Phenyl))-Gly-Asp-Cys-OH;

Ac-Cys-Asn-(DiMeTzl)-(Arg(Benzyl))-Gly-Asp-Cys-OH; and

c(Aha-(homoLys)-Gly-Asp-Trp-Pro).

The preferred compounds are:

Ac-Cys-Asn-(DiMetzl)-(homoLys)-Gly-Asp-Cys-OH; and

c(Aha-(homoLys)-Gly-Asp-Trp-Pro)

In addition to the common three letter abbreviations used to identify common amino acids, applicants have used the following abbreviation designations:

homoLys	homo-lysine
Aha, 7-AhepA	7-aminoheptanoic acid
Arg(Ph)	phenylarginine
Arg(Bzl)	benzylarginine
DiMeTzl	dimethylthioproline
AhexA	6-aminohexanoic acid
AoctA	8-aminooctanoic acid
GuaValA	5-guanidovaleric acid
GuaHexA	6-guanidoxhexanoic acid
GuaHepA	7-guanidoheptanoic acid
beta-Nal	beta-naphthylalanine

The invention also includes compositions, comprising fibrinogen receptor antagonist peptides of the present invention and one or more pharmacologically acceptable carriers, e.g. saline, at a pharmacologically acceptable pH, e.g. 7.4, which are suitable for continuous intravenous or oral or intravenous bolus administration for promoting inhibition of platelet aggregation.

The invention also includes methods for inhibiting platelet aggregation which comprise administering to a patient, either by continuous intravenous or oral or intravenous bolus method, an effective amount of a composition of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Compounds of the invention are fibrinogen receptor antagonists which inhibit fibrinogen induced platelet aggregation. These compounds are prepared by solid phase synthesis which is well known in the art, or by liquid method well known in the art (Neurath, Hill & Boeder, Eds. "The Proteins" 3rd Edition, Vol. II, Academic Press, 1976).

The compounds of the invention are specifically useful for preventing formation of blood clots by inhibiting the binding of fibrinogen to the platelet membrane glycoprotein complex IIb/IIIa receptor. Preferred compounds have selectivity over other integrin receptors, and thus are specifically designed for preventing thrombosis.

The procedures for synthesizing synthetic amino acids defined by XX such as homolysine, 7-aminoheptanoic acid, phenylarginine, 5-guanidovaleric acid, and benzylarginine are well known in the art. Synthesis of DL-homoLys is described, for example, in Payne, Synthetic Comm. 15 (14) , pp. 1277-1290 (1985). Guanylation procedures are described, for example, in Methods of Enzymology, 256,558 (1972), and in A.E. Miller and J.J. Bischoff, Synthesis , pp. 777-779 (1986).

Compounds of the invention may be prepared using solid phase peptide synthesis, such as that described by Merrifield, J. Am. Chem. Soc., 85, 2149 (1964), although other equivalent chemical syntheses known in the art can also be used, such as the syntheses of Houghten, Proc . Natl. Acad. Sci ., 82, 5132 (1985). Solid-phase synthesis is commenced from the C-terminus of the peptide by coupling a protected amino acid to a suitable resin, as generally set forth in U.S. Patent No. 4,244,946, issued Jan. 21, 1982 to Rivier et al., the disclosure of which is h reby incorporated by reference. Solution method can be used as described by Neurath et al. Chapter 2, pp. 106-253. Exampl s of synthesis of this general type are set forth in U.S. Patent Nos. 4,305,872 and 4,316,891.

In synthesizing these polyp ptides, the carboxyl terminal amino acid, having its alpha-amino group suitably protected, is covalently coupled to a chloromethylated polystyrene resin or the like, such as p-

hydroxymethylph nylacetylamidom thylresin (PAM r sin). Th chlorom thylated polystyren resin is composed of fine beads (20-70 microns in diameter) of a synthetic resin prepared by copolymerization of styrene with 1 to 2 percent divinylbenzene. The benzene rings in the r sin are chlorom thylat d in a Friedel-Crafts reaction with chloromethyl methyl ether and stannic chloride. The Friedel-Crafts reaction is continued until the resin contains 0.5 to 5 mmoles of chlorine per gram of resin. After removal of the alpha-amino protecting group, as by using trifluoroacetic acid in methylene chloride, the amino protected derivative of the next amino acid in the sequence is added along with a condensation coupling agent such as dicyclohexylcarbodiimide. The remaining alpha-amino and side-chain-protected amino acids are then coupled by condensation stepwise in the desired order to obtain an intermediate compound connected to the resin.

The condensation between two amino acids, or an amino acid and a peptide, or a peptide and a peptide can be carried out according to the usual condensation methods such as azide method, mixed acid anhydride method, DCC (dicyclohexyl-carbodiimide) method, BOP (benzotriazole-1-yloxytris (dimethylamino) phosphonium hexafluorophosphate method, active ester method (p-nitrophenyl ester method, N-hydroxysuccinimido ester method, cyanomethyl ester method, etc.), Woodward reagent K method, carbonyldiimidazol method, oxidation-reduction method. In the case of elongating the peptide chain in the solid phase method, the peptide is attached to an insoluble carrier at the C-terminal amino acid. For insoluble carriers, those which react with the carboxy group of the C-terminal amino acid to form a bond which is readily cleaved later, for example, halomethyl resin such as chloromethyl resin and bromomethyl resin, hydroxymethyl resin, aminomethyl resin, benzhydrylamine resin, and t-alkyloxycarbonylhydrazide resin can be used.

Common to chemical syntheses of peptides is the protection of the reactive side-chain groups of the various amino acid moieties with suitable protecting groups at that site until the group is ultimately removed after the chain has been completely assembled. Also common is the protection of the alpha-amino group on a amino acid or a fragment while that entity reacts at the carboxyl group followed by the selective removal of the alpha-amino-protecting group to allow subsequent reaction to take place at that location. Accordingly, it is common that, as a step in the synthesis, an intermediate compound is produced which includes each of the amino acid residues located in the desired sequence in the peptide chain with various of these residues having side-chain protecting groups. These protecting groups are then commonly removed substantially at the same time so as to produce the desired resultant product following purification.

The applicable protective groups for protecting the alpha-and omega-side chain amino groups are exemplified such as benzyloxycarbonyl (hereinafter abbreviated as Z), isonicotinyloxycarbonyl (iNOC), 0-chlorobenzyloxycarbonyl [Z(2-Cl)], p-nitrobenzyloxycarbonyl [$Z(NO_2)$],p-methoxybenzyloxycarbonyl [Z-(OMe)],t-butoxycarbonyl (Boc), t-amyloxycarbonyl (Aoc), isobornyloxycarbonyl, adamantyloxycarbonyl, 2-(4-biphenyl)-2- propyloxycarbonyl (Bpoc),9-fluorenylmethoxycarbonyl (Fmoc), methylsulfonylethoxycarbonyl (Msc), trifluoroacetyl, phthalyl, formyl, 2-nitrophenylsulphenyl (NPS), diphenylphosphinothioyl (Ppt), dimethylphosphinothioyl (Mpt) and the like.

Protective groups for carboxy group include, for example, benzyl ester (OBzl), cyclohexyl ester (Chx) 4-nitrobenzyl ester (ONb), t-butyl ester (OBut), 4-pyridylmethyl ester (OPic), and the like. It is desirable that specific amino acids such as arginine, cysteine, and serine possesing a functional group other than amino and carboxyl groups are protected by a suitable protective group as occasion demands. For example, the guanidino group in arginine may be protected with nitro, p-toluenesulfonyl, benzyloxycarbonyl, adamantyloxycarbonyl, p-methoxybenzenesulfonyl, 4-methoxy-2, 6-dimethylbenzenesulfonyl (Mds), 1,3,5-trimethylphenylsulfonyl (Mts), and the like. The thiol group in cysteine may be protected with benzyl, p-methoxybenzyl, triphenylmethyl, acetylamidomethyl, ethylcarbamoyl, 4-methylbenzyl, 2,4,6-trimethylbenzyl (Tmb) etc., and the hydroxyl group in serine can be protected with benzyl, t-butyl, acetyl, tetrahydropyranyl etc.

Stewart and Young, "Solid Phase Peptide Synthesis:, Pierce Chemical Company, Rockford, IL (1984) provides detailed information regarding procedures for preparing peptides. Protection of α-amino groups is described on pages 14-18, and side-chain blockage is described on pages 18-28. A table of protecting groups for amine, hydroxyl and sulfhydryl functions is provided on pages 149-151. These descriptions are hereby incorporated by reference.

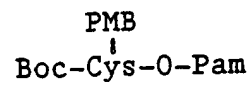
After the desired amino-acid sequence has been completed, the intermediate peptide is removed from the r sin support by treatment with a reag nt, such as liquid HF, which not only cleaves the peptide from th resin, but also cleav s all the r maining protecting groups from the side chain which do not interfere in the cyclization r action. Potentially reactive side chains functionalities ar protected with blocking groups which are stable to HF. The peptides are cyclized by any one of several known procedures (see Schroder and Lubke, "The Peptides: Methods of Peptide Synthesis" Vol. I, Academic Press, New York (1965), pp. 271-286, the contents of which are hereby incorporated by reference), e.g. by forming a disulfide bridge

between the cysteine residues using iodine in AcOH, or air oxidation at pH 8 in dilute NH_4 OAc buffer. The polypeptide can then be purified by gel permeation chromatography followed by preparative HPLC, as described in Rivier et al., Peptides: Structure and Biological Function (1979) pp. 125-128.

EXAMPLE 1

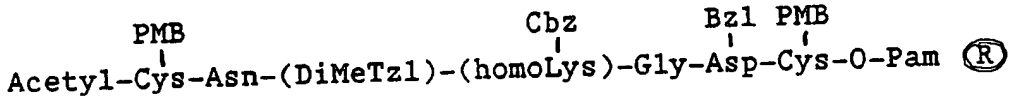
Synthesis of Ac-Cys(Pmb)-Asn-(DiMeTzl)-(homoLys(Cbz))-Gly-Asp(Bzl)-Cys (Pmb)-O Pam Ⓡ and ultimately Ac-Cys-Asn-(DiMeTzl)-(homoLys)-Gly-Asp-Cys-OH

Starting with

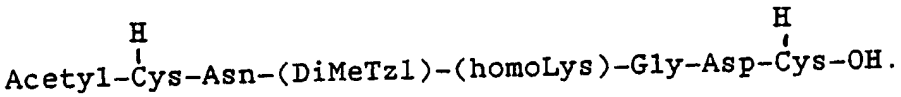


resin, the alpha-amino Boc protecting group (tert-butylcarbonyl) is removed (while the Cys side-chain remains protected by p-methylbenzyl) using trifluoroacetic acid and methylene chloride, and the α-deprotected cysteine neutralized with diisopropylethyl amine. Boc-protected Asp (benzyl) (Asp (Bzl)) is then coupled to cysteine mediated by dicyclohexyl-carbodiimide, and deprotected with trifluoroacetic acid and methylene chloride. Asp is then neutralized with diisopropylethylamine. Following this stepwise procedure of coupling with dicyclohexylcarbodiimide, deprotection with trifluoroacetic acid and methylene chloride, and neutralization with diisopropylethylamine, Boc-protected Gly, homoLys(Cbz) DiMeTzl, Asn, Cys(Pmb) residues are coupled in succession. The final Cys is then acetylated with acetic anhydride.

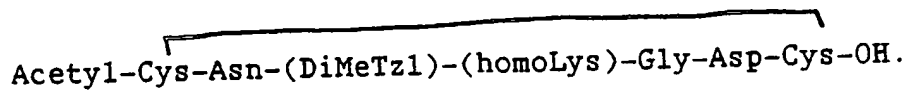
Following acetylation, the following peptide-resin is formed:



Cleavage of the peptide from the resin is achieved using HF/anisole (9:1 (v/v)) to form:



A cyclic structure is formed by formation of a disulfide bridge between the cysteine residues. The peptide is dissolved in 50-80% AcOH:H_2O at room temperature, and the solution stirred during rapid addition of a solution of iodine in AcOH to a final concentration of 2.25 mg/ml of iodine. After 1-2 hours reaction time, excess I_2 and AcOH are removed by rotary evaporation under vacuum and the aqueous solution containing the cyclized peptide is purified using preparative HPLC in 0.1% TFA H_2O-CH_3CN gradient at which stage the D- and L- diastereomers are separated by conventional means. The final TFA salt product is converted to HOAc salt by passing through an ion exchange column BioRad AG3-X4A (acetate cycle). The finished peptide is:



As an alternative to formation of the disulfide by iodine oxidation, the free SH peptide is dissolved in 1-5% HOAc at a concentration of approximately 2 mg/ml and the solution is adjusted to approximately pH 7-8.5 with concentrated NH_4OH. Cyclization is accomplished under brisk stirring (preferably with a small piece of copper wire add d to accelerate the reaction) during a period of 1-4 hours at 25°. The reaction mixture is then concentrated as before and product purified by pr parative HPLC.

Therapeutic Utility

Compounds of the invention may be administered to patients where prevention of thrombosis by inhibiting binding of fibrinog n to the platelet membran glycoprotein complex IIb/IIIa receptor is desired. They are useful in surgery on p ripheral arteries (arterial grafts, carotid endarterectomy) and in cardiovascular surgery where manipulation of arteries and organs, and/or the interaction of platelets with artificial surfaces, leads to platelet aggregation and consumption. The aggregated platelets may form thrombi and thromboemboli. Polypeptides of the invention may be administered to these surgical patients to prevent the formation of thrombi and thromboemboli.

Extracorporeal circulation is routinely used for cardiovascular surgery in order to oxygenate blood. Platelets adhere to surfaces of the extracorporeal circuit. Adhesion is dependent on the interaction between GPIIb/IIIa on the platelet membranes and fibrinogen adsorbed to the surface of the circuit. (Gluszko et al., Amer. J. Physiol., 1987, 252:H, pp 615-621). Platelets released from artificial surfaces show impaired hemostatic function. Polypeptides of the invention may be administered to prevent adhesion.

Other applications of these polypeptides include prevention of platelet thrombosis, thromboembolism and reocclusion during and after thrombolytic therapy and prevention of platelet thrombosis, thromboembolism and reocclusion after angioplasty of coronary and other arteries and after coronary artery bypass procedures. Polypeptides of the invention may also be used to prevent myocardial infarction.

These polypeptides may be administered by any convenient means which will result in its delivery into the blood stream in substantial amount including continuous intravenous or bolus injection or oral methods. Compositions of the invention include peptides of the invention and pharmacologically acceptable carriers, e.g. saline, at a pH level e.g. 7.4, suitable for achieving inhibition of platelet aggregation. They may be combined with thrombolytic agents such as plasminogen activators or streptokinase in order to inhibit platelet aggregation. They may also be combined with anticoagulants such as heparin, aspirin or warfarin. Intravenous administration is presently contemplated as the preferred administration route. They are soluble in water, and may therefore be effectively administered in solution.

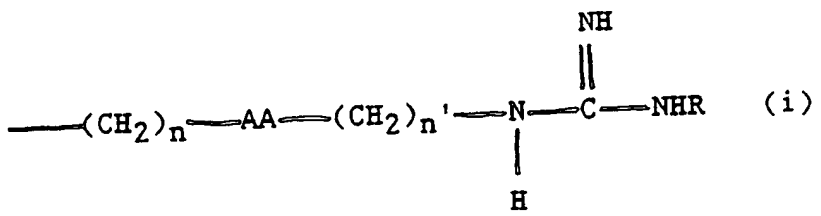
In one exemplary application, a suitable amount of peptide is intravenously administered to a heart attack victim undergoing angioplasty. Administration occurs during or several minutes prior to angioplasty, and is in an amount sufficient to inhibit platelet aggregation, e.g. an amount which achieves a steady state plasma concentration of between about 0.05-30 μM per kilo, preferably between about 0.3-3 μm per kilo. When this amount is achieved, an infusion of between about 1-100 nM per kilo per min., preferably between about 10-30 nM per kilo per min. is maintained to inhibit platelet aggregation. Should the patient need to undergo bypass surgery, administration may be stopped immediately and will not cause complications during surgery that would be caused by other materials such as aspirin or monoclonal antibodies, the effects of which last hours after cessation of administration.

The present invention also includes a pharmaceutical composition comprising peptides of the present invention and tissue type plasminogen activator or streptokinase. The invention also includes a method for promoting thrombolysis and preventing reocclusion is a patient which comprises administering to the patient an effective amount of compositions of the invention.

The present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof. Thus, the specific examples described above should not be interpreted as limiting the scope of the present invention.

Claims

1. A fibrinogen receptor antagonist compound which comprises the sequence
XX-Gly-Asp
wherein XX represents a synthetic alpha-amino acid containing a linear side chain defined as



(i)

or

$-(CH_2)_n$-AA-$(CH_2)_{n'}$-NHR (ii)

wherein:

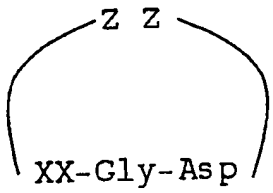
n is 1,2,3 or 4;

n′ is 2,3 or 4;

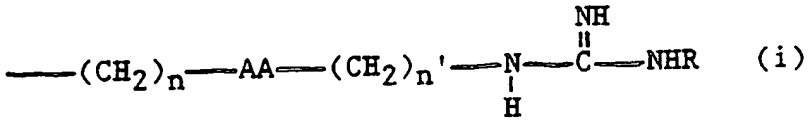
AA is an oxygen atom, a sulfur atom, or a single bond; and

R is H, C_{1-6} alkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylmethyl or substituted or unsubstituted cycloalkyl, provided that in case (i), when AA is a single bond and R is H, then n+n′ does not equal 1, 2, 3 or 4.

2. A fibrinogen receptor antagonist of the formula:



wherein XX represents a synthetic alpha-amino acid having a side chain containing a linear side chain defined as



(i)

or

$-(CH_2)_n$-AA-$(CH_2)_{n'}$-NHR (ii)

wherein:

n is 1,2,3 or 4;

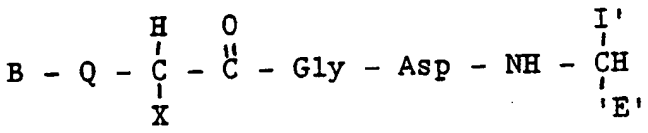
n′ is 2,3 or 4;

AA is an oxygen atom, a sulfur atom, or a single bond; and

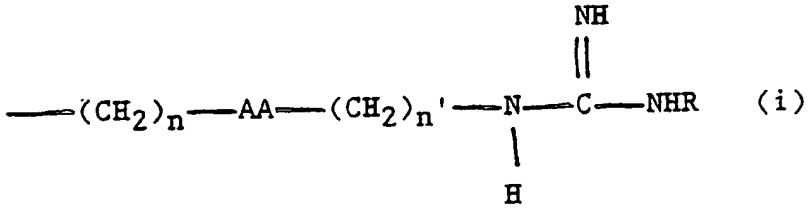
R is H, C_{1-6} alkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylmethyl or substituted or unsubstituted cycloalkyl, provided that in case (i), when AA is a single bond and R is H, then n+n′ does not equal 1, 2, 3 or 4,

and ZZ represents a sequence of 1, 2, 3 or 4 substituted or unsubstituted amino acids.

3. A fibrinogen receptor antagonist compound of the formula:



wherein B represents zero, one or two substituted or unsubstituted amino acids; Q represents H,NH,NH_2, or Ac-NH; X represents an amino acid side chain defined as



(i)

or

$-(CH_2)_n-AA-(CH_2)_{n'}-NHR$ (ii)

wherein:

n is 1,2,3 or 4;

n' is 2,3 or 4;

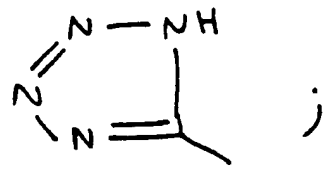
AA is an oxygen atom, a sulfur atom, or a single bond; and

R is H, C_{1-6} alkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylmethyl or substituted or unsubstituted cycloalkyl, provided that in case (i), when AA is a single bond and R is H, then $n+n'$ does not equal 1, 2, 3 or 4,

and

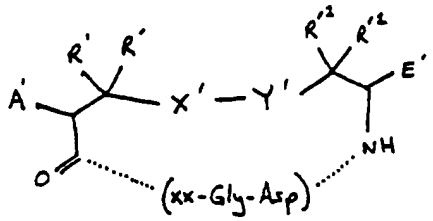
I' represents a side chain of an L-amino acid,

E' is H, COOH, $CONH_2$, $CONHR^2$, $CONR^3R^4$, CH_2OH,CO_2R^2,CH_3 wherein R^2 is an alkyl group having 1 to 4 carbon atoms, R^3R^4 is an alkyl group having 1 to 4 carbon atoms or NR^3R^4 is a secondary amino acid, or

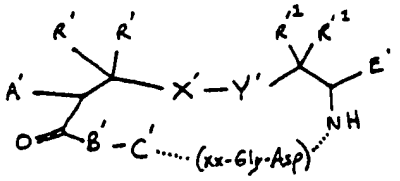


provided that when B is zero substituted or unsubstituted amino acids, then Q is H,NH_2 or Ac-NH, and that when B is one or two substituted or unsubstituted amino acids, then Q is NH.

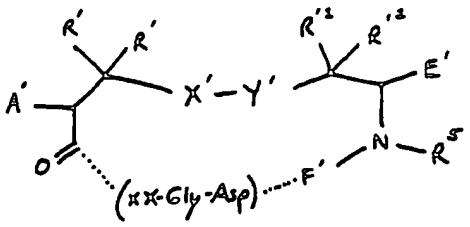
4. A compound of claim 2 wherein ZZ is 1, 2, 3 or 4 amino acids according to formulas I, II, III, IV or V:



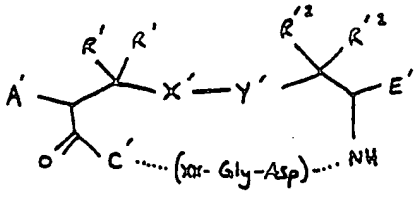
(I)



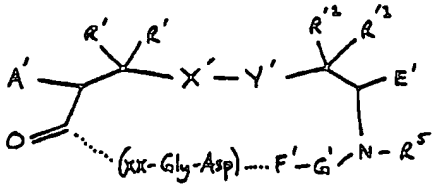
(II)



(III)

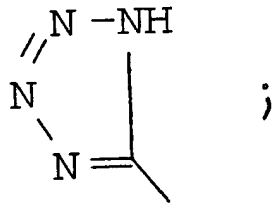


IV



V

wherein
A′ is H, acylamido, acylaminoacylamido, acylamino-N-methylaminoacyl-amido;
R′ and R′¹ are independently H, methyl, ethyl or a lower alkyl group having 1 to 5 carbons;
X′-Y′ is S-S, CH_2-S, S-CH_2, CH_2CH_2, CH_2,$CH_2CH_2CH_2$, CH_2-S-S, CH_2-S-S-CH_2, S-S-CH_2; and
E′ is H, COOH, $CONH_2$, $CONHR^2$, $CONR^3R^4$, CH_2OH,CO_2R^2,CH_3 wherein R^2 is an alkyl group having 1 to 4 carbon atoms, R^3R^4 is an alkyl group having 1 to 4 carbon atoms or NR^3R^4 is a secondary amino acid, or



;

B′ is a D- or L- α-amino acid;
C′ is a D- or L- secondary α-amino acid or a D- or L-primary amino acid;
F′ is an L- α-mino acid;
G′ is a D- or L- α-amino acid, secondary cyclic amino acid, or N-methyl amino acid; and
R5 is H or methyl.

5. A compound of claim 2 which is

Ac-Cys-Asn-(DiMeTzl)-(homoLys)-Gly-Asp-Cys-OH

6. A compound of Claim 1 which is
c(Aha-(homoLys)-Gly-Asp-Trp-Pro)
7. A composition for inhibiting fibrinogen-depend nt platelet aggregation in a mammal comprising a compound of claim 1 and a pharmaceutically acceptable carrier.
8. The use of a compound of Claim 1 for the preparation of a medicament suitable for inhibiting fibrinogen binding to mammalian platelets.
9. A composition for inhibiting fibrinogen-dependent platelet aggregation in a mammal comprising a compound of claim 2 and a pharmaceutically acceptable carrier.
10. The use of a compound of Claim 2 for the preparation of a medicament suitable for inhibiting fibrinogen binding to mammalian platelets.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 31 1151

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-0 275 748 (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE (INSERM))		C 07 K 15/00

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 07 K

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	22 January 91	DEFFNER C-A.E.

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same catagory
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention

E: earlier patent document, but published on, or after the filing date
D: document cited in the application
L: document cited for other reasons

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&: member of the same patent family, corresponding document